Identifying a type of genetic code in an anonymous, prokaryotic DNA sequence

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Introduction

- It was commonly believed that genetic code was universal when it was discovered.
- Due to technological advances variations of the canonical code have been discovered.
  → Calling for an *ab-initio* approach
- Recently, phages with two different genetic codes have been described.
  → Computational tool must be able to predict potential switching points

Why is gene prediction important?

- If an outbreak of a new virus has happened:
  → accurate gene prediciton is required to help to identify potential drug targets in downstream analysis

Conclusion

- First tool of its kind
- Accurate on complete genomes and contigs greater than 10Kbp
  → makes use of other codon frequencies to determine to which amino acid a stop codon is reassigned
- Code switch point predicted with a mean error of 0.53 genes ± 6.47 genes
  → Utilizes prediction of canonical and non-canonical model to refine switching point predictions in difficult cases

Contact

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References

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2. A. Rodin and S. Branciamore, The Universal Genetic Code and Non-Canonical Variants
3. N. N. Ivanova et al., Stop codon reassignments in the wild
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5. E. Guerin et al., Biology and Taxonomy of crAss-like Bacteriophages, the Most Abundant Virus in the Human Gut
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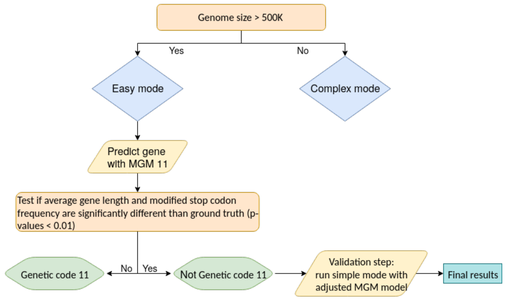
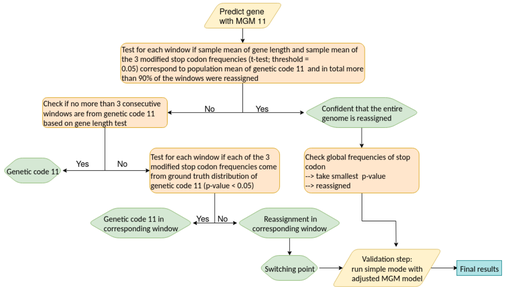
Materials and Methods

Workflow of Genetic Code Identifier

Simple mode



Complex mode



Data

- Dataset 1: 100 representative genomes with genetic code 4 and 25; GC%: 20%-40%; Size: <= 2.25Mbp
- Dataset 2: 95 representative genomes with genetic code 11; GC%: 20% - 35%; Size <= 2Mbp
- Dataset 3: 100 representative genomes with genetic code 11; GC%: 20% - 80%; Size <= 12Mbp
- Dataset 4: +5000 representative genomes used for the training of GeneMark-S2
- Dataset 5: 42 genomes of phages; GC%: 27% - 35%; Size: 175Kbp

Features

1. **Modified stop codon frequencies**

$$f(TAA)' = f(TAA) \times GC\% \quad (1a)$$
$$f(TGA)' = f(TGA) \times (1 - GC\%) \quad (1b)$$
$$f(TAG)' = f(TAG) \times (1 - GC\%) \quad (1c)$$

- If reassigned: frequency is significantly increased

2. **Average gene length**

- MetaGeneMark model with correct genetic code ~1000nt
- MetaGeneMark model with incorrect genetic code ~400nt

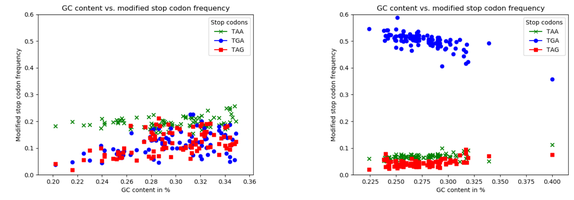


Figure 1: Modified Stop codon frequencies. TGA is reassigned in genetic code 4, its frequency is significantly increased.

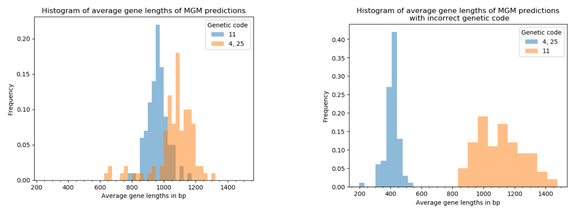


Figure 2: Av. gene lengths drops from 1000nt to 400nt if incorrect model is employed (dataset 1& 2).

Results

Simple mode	Data 1	Data 2	Data 3
No reassignment	0	95	100
Complete reassignment	100	0	0
Accuracy	1.0	1.0	1.0
Complex mode	**Data 1**	**Data 2**	**Data 3**
No reassignment	0	88	99
Partial reassignment	1	7	1
Complete reassignment	99	0	0
Accuracy	0.99	0.93	0.99

Table 1: Results on dataset 1, 2 & 3. No misclassifications were made in the simple mode. When employing the complex mode there is one genome predicted to have a partial reassignment of ~85% in dataset 1. In dataset 2 & 3 some partial reassignments of less than 10% are predicted and hence should be considered as artifacts.

1. Simple mode

- Tested on **dataset 4**:
  - 5 genomes annotated as genetic code 4 but predicted with genetic code 11 at NCBI
  - All *Acholeplasma sp.*
    - Av. Gene length ~1000nt
      → NCBI **agreed and changed** code assignment

2. Complex mode

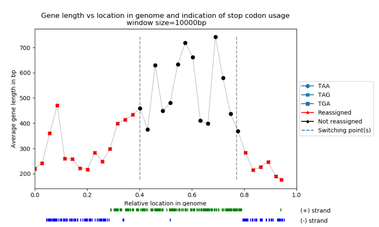


Figure 3: The analysis iof a genome of a phage in **dataset 5**. The phage is predicted to have two different genetic codes. The predictions are concordant with the literature.

- Split dataset 1 & 2 into contigs of different sizes and evaluated performance

$$\left.\begin{aligned} Specificity &= 0.99 \\ Sensitivity &= 0.92 \end{aligned}\right\} contigs\ of\ size\ 10Kbp$$

- Merged contigs from dataset 1 & 3 to simulate switching points
  - **Mean error** 0.53 genes ± 6.47 genes
  - in reality it might be less when strand information can be utilized for refinement of the prediction
    → simulated genomes do not show change in encoding as observed in the phages of dataset 5

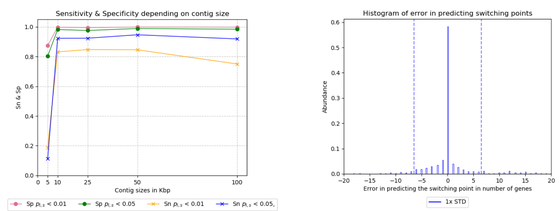


Figure 4: Evaluation of the complex mode and the switch point prediction on simulated contigs.